REMARKS

Claims 1-23 were present in the application as filed. In response to a first Office Action, claims 2-4 were cancelled and new claim 24 was added. Claims 1 and 5-24 were pending in the application. Claims 18 and 19 are cancelled above; claims 1, 5-17 and 20-24 remain pending in the application. Reconsideration of the amended claims is respectfully requested.

Claim 1 is amended above to focus the claim on a method for determining the formation of endothelins in patients suspected of having cardiovascular disease or sepsis. Related to this amendment, claim 16 is amended to recite specific cardiovascular diseases, for example, pulmonary arterial hypertension, atherosclerosis, heart failure, and myocardial infarction. Support for the amendment can be found in the specification at page 3, lines 27-32 (Also see attached information from Wikipedia and American Heart Association with regard to various conditions which come under the umbrella of Cardiovascular Disease.)

Rejection Under 35 U.S.C. §112, second paragraph

Claims 1, 5-19 and 24 are rejected under 35 U.S.C.§112, second paragraph, as being indefinite. According to the Office Action, the recitation of "a C-terminal fragment detected" is vague and indefinite because it is unclear if Applicant is referring to the C-terminal fragment detected earlier in the claims or if Applicant intends another C-terminal fragment.

The claims are amended above to recite "the C-terminal fragment" to indicate that the fragment is the same as the earlier recited C-terminal fragment that is detected by the antibody pair.

Claim 1 is also allegedly indefinite because, according to the Office Action, it is unclear how the formation of endothelin-1 and big endothelin-1 is determined because the claim is directed to the detection of C-terminal fragments of preproendothelin-1.

Preproendothelin-1 is a prohormone, the processing of which will ultimately give rise to the vasoactive hormone, endothelin-1. Enzymatic processing of preproendothelin-1 at specific cleavage sites yields proendothelin-1, which is further cleaved to big endothelin-1; big endothelin-1 is further cleaved to produce endothelin-1. What remains of the prohormone is a C-terminal portion with no known function. This C-terminal portion, however, persists in the circulation after the active hormone has been cleared. There is a stoichiometric relationship between endothelin-1 and the C-terminal fragment, that is, from each preproendothelin molecule one endothelin-1 and one C-terminal fragment is generated, making the C-terminal fragment a suitable surrogate marker for the formation of endothelin-1.

Furthermore, when an antibody against a region further downstream from the 168-212 region (that is toward the N-terminus), specifically, an antibody generated against a peptide consisting of amino acids 32-52 or 136-148 of preproendothelin-1, was substituted for one of the anti-168-212 region antibodies, it was determined that intact preproendothelin-1 was not present in the samples tested; it is likely that the 93-212 C-terminal of preproendothelin remaining after cleavage of endothelin-1 undergoes additional processing (page 23, lines 23-29.)

Withdrawal of the rejection under 35 U.S.C.§112, second paragraph is respectfully requested.

Rejection Under 35 U.S.C. §112, first paragraph

Claims 1, 5-19 and 24 are rejected under 35 U.S.C.§112, first paragraph, as failing to satisfy the enablement requirement. According to the Office Action, the specification, while "being enabling for a method for detecting C-terminal fragments of preproendothelin-1 (SEQ ID NO: 1)…by contacting the sample with antibodies which specifically bind within amino acids 169-181, 184-203 and 200-212 of preproendotherlin-1, does not reasonably provide enablement for any and all antibodies and any and all sequence positions within amino acids 168-212 of preproendothelin-1 or detection in any inflammatory or cancer condition." According to the Office Action, enablement requires that the specification teach those in the art to make and use the invention without undue experimentation.

The invention, as claimed, is directed to a method for the determination of the formation of endothelins in a patient in whom certain cardiovascular diseases, or sepsis is suspected, based on the level of a C-terminal fragment of preproendothelin-1 in said sample using first and second antibodies that bind to a specific region of preproendothelin-1, namely, amino acids 168-212 of preproendothelin-1.

Enablement

As discussed previously in Applicants' response of record, an application satisfies the enablement requirement if one skilled in the art, after reading the disclosure, could practice the invention claimed without undue experimentation. *In re Wands, 858 F.2d 731.* The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation "must not be unduly extensive." *Chiron Corporation v. Genentech, Inc., 363 F.3d 1247.* Only after it has been determined that additional experimentation is required, does an analysis under the Wands factors, of whether that experimentation is undue, become applicable. In the present case, no additional experimentation is required before the skilled artisan can practice the claimed method based on the guidance provided in the specification.

Preliminarily, a patent disclosure need not enable information within the knowledge of an ordinary artisan. In the instant case, methodology required for the detection of a protein such as preproendothelin-1 or a fragment thereof, for example, a dual antibody immunoassay method for detecting the presence of a purported biomarker in a biologically relevant tissue or sample, is well known to those of skill in the art. Similarly, methods for the production and affinity purification of anti-peptide antibodies are routine. Measurement of protein biomarkers, routinely done by analysis of a blood sample, such as whole blood, plasma or serum is also known in the art. Thus, in order to enable the claimed method, Applicants are required to (1) identify the patient population to be tested, (2) identify the particular region of preproendothelin-1 that is clinically significant and to which antibodies are made; and (3) provide a correlation between a disease state (shown in Figures 3 and 4) and the presence of a reliable biomarker (a

preproendothelin-1 fragment comprising amino acids 168-212) in a significant number of patients.

1. Patient Population

Claim 1 is amended above to focus the method on specific patient populations, i.e., those in whom sepsis or cardiac disease is suspected.

2. Anti-Peptide (168-212) Antibodies

In order to determine the level of endothelin-1 production in certain enumerated disease states, Applicants hypothesized that, instead of measuring the active peptide itself, which is rapidly cleared, they could measure the portion of the precursor molecule, preproendothelin-1, which remains once endothelin-1 is cleaved; as it turns out, the remainder of the preproendothelin-1 molecule persists in circulation long after the active peptide, endothelin-1, is cleared.

Applicants identified a C-terminal fragment of preproendothelin-1 comprising amino acids 168-212 that is informative of the level of endothelin-1 formed in a disease state such as cardiac disease or sepsis.

A suitable dual antibody immunoassay for the measurement of these C-terminal fragments of preproendothelin-1 would, therefore, utilizes a pair of antibodies directed to the 168-212 region of preproendothelin-1 and only to that region. Using methodology that is routine in the art for immunization, screening, affinity purification of antibodies, etc., the skilled artisan would have a reasonable expectation of producing two different antibodies that would bind the 168-212 region of preproendothelin-1.

Polyclonal antibodies raised against three peptide immunogens, PCT15 (amino acids 168-181), PNR20 (amino acids 184-203) and PCW14 (amino acids 200-212) are exemplified. The specification discloses that the combination of the anti-168-181 and anti-200-212 antibodies gave the expected result and that substitution of one of those antibodies with the anti-184-203 antibody gave the identical result (page 23, lines 11-16.) Applicants, therefore, have identified three peptides within the 168-212 region, which are able to elicit antibodies that not only bind to

the 168-212 region, but when paired together, are able to accurately detect the level of a C-terminal fragment comprising amino acids 168-212 of preproendothelin-1.

The Office Action maintains that the specification fails to teach any and all antibodies that bind to any and all sequence combinations of amino acids 168-212 of preproendothelin-1 and does not provide examples of epitopes or antibodies to such combinations, for example, amino acids 168-169, 169-170, 169-179 etc.

As a practical matter, one of skill in the art would recognize that not every amino acid, or linear sequence of amino acids constitutes an epitope. Typically, a minimal epitope is about 5-7 amino acids in length. Furthermore, Applicant is not required to enable every possible embodiment to satisfy the enablement requirement. Nonetheless, Applicants' method does not require antibodies to specific epitopes within the 168-212 region, rather, the method of the invention requires any pair of antibodies (capture and detection antibodies) that both bind to the 168-212 region of preproendothelin-1. Having identified the region of preproendothelin-1 which is the target to be detected (i.e., the 168-212 region of preproendothelin-1), and three peptides that are cabable of generating appropriate antibodies, one of skill in the art would have a reasonable expectation that by using a peptide immunogen having an amino acid sequence derived from that region she would obtain antibodies that bind to that region.

3. Correlation Between Marker and Disease State

A correlation between disease state and elevated levels of the purported biomarker (a preproendothelin-1 fragment comprising amino acids 168-212) in a significant number of patients is shown in Figures 3 (atherosclerosis, heart failure and cardiac infarction) and 4 (severe sepsis and septic shock).

Applicants, therefore, have met their burden. The level of guidance found in the specification with respect to the patient population to be tested, assay to be used and antibodies to be used is such that no additional guidance is necessary and no undue experimentation is required for one of skill to practice the claimed method.

Withdrawal of the rejection under 35 U.S.C. §112, first paragraph is respectfully requested.

In view of the foregoing amendments and remarks, the application is now in condition for allowance and reconsideration and prompt allowance are respectfully requested. The Examiner is encouraged to contact Applicants' undersigned representative if clarification of any of the above remarks would be helpful in placing the case in better condition for allowance.

Respectfully submitted,

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